"On the Discovery of a Species of Trypanosoma in the Cerebro-Spinal Fluid of Cases of Sleeping Sickness." By ALDO CASTELLANI, M.D. Communicated by the Malaria Committee of the Royal Society. Dated "Entebbe, Uganda, 5th April, 1903." Received May 8,—Read May 14, 1903.

On the 12th November, 1902, when examining a specimen of cerebro-spinal fluid taken by lumbar puncture during life from a well-marked case of *sleeping sickness*, I was surprised to observe a living trypanosoma. Since that date I have made as many observations in this direction as possible, and the results are to my mind sufficiently surprising to excuse me for presenting this preliminary note.

These trypanosomes are not in large numbers, so that to find them it is necessary to draw off at least 15 c.cs. of the cerebro-spinal fluid. It is better to reject the first few c.cs. as they are apt to contain blood. When the fluid comes away clear, 10 c.cs. are collected and centrifuged for 15 minutes. At the end of this time there is found at the bottom of the tube a slight deposit of whitish sediment, and in some cases also a minute trace of blood.

The liquid above the sediment is poured off and the sediment examined under a moderately low power of the microscope. As the trypanosomes are at first fairly active they are easily detected.

The following tables represent the results of this investigation:—

Table I.—Sleeping Sickness Cases.

No.	Name.	Sex.	Age.	Date.	Stage of disease.	Microscopic appearance of sediment.	Presence of trypanosoma in cerebro-spinal fluid.	Remarks.
1	Mundo	M.	15	12/11/02	3rd	A few leucocytes, the majority of which are mononuclear. Some very rare red blood corpuscles	Present	
2	Maoli.....	M.	18	25/11/02	3rd	Some few leucocytes and R.B.C.	Absent	
3	Aritzo	M.	25	7/12/02	3rd	Some leucocytes. No R.B.C.	Absent	
4	Manika	F.	10	15/12/02	3rd	A few leucocytes. Very few R.B.C.	Present	Patient died on December 18, 1902. No complications. In fluid from lat. vent. Tryp. present.
5	Ialika	F.	22	15/12/02	2nd	A few leucocytes. Very few R.B.C.	Absent	
6	Asmeni	F.	8	15/12/02	3rd	Some leucocytes and many R.B.C.	Absent	
7	Bolenti	M.	10	22/12/02	3rd	Some leucocytes. No R.B.C.	Present	In fresh preparations of blood taken from a finger the same day I found a few trypanosomes apparently similar to those found in the lumbar puncture liquid, only their movements were apparently more lively.
8	A	M.	20	5/1/03	3rd	Few leucocytes and some R.B.C.	Absent	
				7/1/03	..	Some leucocytes and very few R.B.C.	Absent	

Table I.—Sleeping Sickness Cases—*continued.*

No.	Name.	Sex.	Age.	Date.	Stage of disease.	Microscopic appearance of sediment.	Presence of trypanosoma in cerebro-spinal fluid.	Remarks.
9	Makassa	F.	25	25/1/03	3rd	Some leucocytes. No R.B.C.	Present	
10	Kaperi II	M.	14	25/1/03	2nd	A few leucocytes and very few R.B.C.	Absent	
11	Ally II	M.	30	2/2/03	3rd	Some leucocytes and R.B.C.	Absent	
12	Mocreza	M.	30	10/2/03	2nd	A few leucocytes and R.B.C.	Absent	
13	Budara	M.	22	27/2/03	2nd	Some leucocytes and very few R.B.C.	Present	
				2/3/03	..	Some leucocytes and very few R.B C.	Present	
14	Nombi	F.	30	27/2/03	1st	Few leucocytes and R.B.C.	Absent	
				24/3/03	2nd	Some leucocytes and R.B.C.	Absent	
15	Fatoma	F.	18	27/2/03	2nd	Very scarce leucocytes and no R.B.C.	Absent	
				4/3/03	..	Few leucocytes and R.B.C.	Absent	
				26/3/03	..	Few leucocytes and R.B.C.	Absent	
				1/4/03	..	Some leucocytes and a few R.B.C.	Present	
16	Zenabu	F.	22	24/3/03	1st	No R.B.C.	Absent	
				28/3/03	1st	Some R.B.C.	Absent	
				1/4/03	1st	Some RB.C.	Absent	

Table I.—Sleeping Sickness Cases—*continued.*

No.	Name.	Sex.	Age.	Date.	Stage of disease.	Microscopic appearance of sediment.	Presence of trypanosoma in cerebro-spinal fluid.	Remarks.
17	Benjamin	M.	20	25/3/03	2nd	..	Absent	
				28/3/03	..	Some few leucocytes and R.B.C.	Absent	
				29/3/03	..	Some few leucocytes and R.B.C.	Absent	
18	Zakibu	M.	25	25/3/03	2nd	Few leucocytes and R.B.C.	Present	
				27/3/03	2nd	Few leucocytes and R.B.C.	Present	
19	Seera	M.	25	26/3/03	2nd	..	Present	
20	Kimbra	M.	55	26/3/03	3rd	Some leucocytes. No R.B.C.	Present	
21	Abdulla	M.	..	26/3/03		No R.B.C.	Present	
22	Kagoya	F.	20	26/3/03	3rd	No R.B.C.	Present	
23	Keogaffum	M.	55	27/3/03	2nd	Some leucocytes. No R.B.C.	Absent	
				1/4/03		..	Absent	
24	Jacobo	M.	20	28/3/03	3rd	Some leucocytes. No R.B.C.	Present	The trypanosomes were much more numerous than in other cases.
25	Iegobaza	M.	40	27/3/03	2nd	Few leucocytes. No R.B.C.	Present	
26	Ibsarara	F.	35	27/3/03	3rd	Some leucocytes. No R.B.C.	Present	
27	Leobeni	M.	25	28/3/03	2nd	No. R.B.C.	Present	
28	Kidorme	M.	20	28/3/03	2nd	Some leucocytes and R.B.C.	Present	

Table I.—Sleeping Sickness Cases—*continued.*

No.	Name.	Sex.	Age.	Date.	Stage of disease.	Microscopic appearance of sediment.	Presence of trypanosoma in cerebro-spinal fluid.	Remarks.
29	Kcagabidoia	M.	55	28/3/03	3rd	Some leucocytes and R.B.C.	Absent	
				1/4/03	3rd	Some leucocytes and R.B.C.	Absent	
30	Kitaroma	M.	25	28/3/03	2nd	Some leucocytes and R.B.C.	Absent	Only 5 c.c. of liquid collected.
				2/4/03	2nd	Some leucocytes and R.B.C.	Absent	
31	Waiswa	M.	10	29/3/03	2nd	Some leucocytes and R.B.C.	Present	
32	Kaperi I.	M.	8	23/3/03	3rd	No. R.B.C.	Present	Trypanosoma present also in the fluid taken from lateral vent. at the *post-mortem*.
33	Matasa	M.	28	29/3/03	2nd	Few leucocytes and R.B.C.	Absent	
				2/4/03	2nd	Few leucocytes and R.B.C.	Absent	
34	Decodeno	M.	25	31/3/03	2nd	Few leucocytes and R.B.C.	Present	

Table II.—Controls.

No.	Name.	Sex.	Age.	Date.	Disease.	Microscopic appearance of sediment.	Presence of trypanosoma.	Remarks.
1	Doanira	M.	35	11/1/03	Chronic nephritis	Very few leucocytes and R.B.C.	Absent	
2	Kano Buringo	M.	30	24/3/03	Trypanosoma fever	Very few leucocytes and R.B.C.	Absent	Dr. Baker found *Trypanosoma Gambiense* in blood some days ago.
3	Landu	M.	..	24/3/03	Enlargement of femoral glands	Few leucocytes. No R.B.C.	Absent	
4	Kamsuro	M.	..	28/3/03	Trypanosoma fever	Few leucocytes and R.B.C.	Absent	Dr. Baker found *Trypanosoma Gambiense* in blood the same morning.
5	Zake	M.	25	30/3/03	Cellulitis	Few leucocytes. No R.B.C.	Absent	
6	Scongo.....	..	..	30/3/03	Itch	Very few leucocytes and R.B.C.	Absent	
7	Pio.....	M.	12	30/3/03	..	Very few leucocytes and R.B.C.	Absent	
8	Kaperi III.....	M.	25	30/3/03	Itch	Very few leucocytes and R.B.C.	Absent	
9	Eliza.....	F.	18	30/3/03	Yaws	Very few leucocytes and R.B.C.	Absent	
10	Bofralour.....	M.	16	31/3/03	Pleuritis	Very few leucocytes and R.B.C.	Absent	
11	Zanabu II	F.	30	31/3/03	Headache	Very few leucocytes and R.B.C.	Absent	
12	Jordien Murjan...	M.	35	31/3/03	Trypanosoma fever.	Very few leucocytes and R.B.C.	Absent	Dr. Baker found *Trypanosoma Gambiense* in blood the same morning.

Table I shows that in 34 cases of sleeping sickness, the trypanosomes were found in the cerebro-spinal fluid taken by lumbar puncture during life in 20 cases, giving a rate of 70 per cent.

On two occasions I also examined in the same way fluid from the lateral ventricles and in both cases found the same parasite. In blood I found the trypanosoma once with certainty.

It may be thought that the trypanosomes are found in the cerebro-spinal fluid on account of the trace of blood which sometimes forms part of the sediment. But it will be seen from the table that in several cases there was no trace of blood.

Table II shows that in 12 cases of ordinary disease, the cerebro-spinal fluid taken during life by lumbar puncture, in no case contained trypanosoma, and it is important to note that 3 of these controls were cases of the usual trypanosoma fever, as described by Forde, Dutton, Manson, Daniels, &c.

Here it may be remarked that trypanosoma fever is by no means uncommon among the natives in Uganda, 3 cases having been met with, by Dr. Baker, one of the colonial surgeons here (Entebbe), within the last 3 weeks. I understand that Dr. Baker is publishing this most interesting observation. It must be clearly understood that these cases of trypanosoma fever bear no resemblance in their clinical features to sleeping sickness.

The trypanosoma found in the cerebro-spinal fluid of sleeping sickness does not as far as I have been able to make out differ materially in size and shape from the species one finds in the blood of trypanosoma fever, *Trypanosoma Gambiense* (Dutton), but possibly it is to be differentiated from this one, because in it, as a rule, the micro-nucleus lies nearer the extremity and the vacuole is apparently larger. Besides, its movements are not apparently so active, but this fact might be due to the effects of the centrifuge. In case it should prove to be a new species, the trypanosoma I have described might be called from the country where I have found it first—*Trypanosoma Ugandense.*

Relation of the Trypanosoma to Sleeping Sickness.

At the post-mortem examination of 80 per cent. of the cases where I found during life the trypanosoma, I grew from the blood of the heart and from the liquid of the lateral ventricles the variety of streptococcus I described many months ago in my first note. Up to that time I had never found the trypanosoma, but this is easily explained by the fact that I did not use the technique I have described in this note, viz., examination of a *large* quantity of liquid after long use of the centrifuge.

Influenced by my last investigations I would suggest as a working hypothesis on which to base further investigation that sleeping sickness

is due to the species of trypanosoma I have found in the cerebro-spinal fluid of the patients in this disease, and that at least in the last stages there is a concomitant streptococcus infection which plays a certain part in the course of the disease.

Note by the Secretary of the Royal Society.

As so far supporting the observations by Dr. Castellani recorded in the above communication, it may be desirable to state that Colonel Bruce, to whom in Uganda Dr. Castellani made known his discovery of the Trypanosoma, and who is now continuing the investigation begun by Dr. Castellani, has sent to the Royal Society a telegram, received May 4, stating that since Dr. Castellani left, in thirty-eight cases of sleeping sickness, he had found trypanosoma in every case in fluid obtained by lumbar puncture, and that he had found trypanosoma in the blood in twelve out of thirteen cases of sleeping sickness.

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